REMARKS

Claims 1, 3-13, and 25-30 are pending in the present application. Applicant has cancelled claims 2, 14-24, and 31-36. Applicant has amended claims 3, 10, 12, and 26 to clarify language and correct antecedent basis. No new matter is added by the amendments.

The Invention

Claims 1, 3-5, 10-13, and 25-30 are drawn to a method of preparing a lactase microcarrier (e.g., independent claim 1) and a permeabilized bacterium containing a heterologous beta-galactosidase, and exhibiting beta-galactosidase activity of at least 4,000 Miller units (e.g., independent claim 25). The method of the invention includes the steps of transforming a food-grade lactic acid bacterium with a DNA construct containing a promoter that is operatively linked to a DNA sequence encoding a beta-galactosidase (BG), culturing the bacterium under conditions that enable expression of the BG such that the bacterium exhibits a BG activity of at least 4,000-10,000 Miller Units (MU), and permeabilizing the bacterium. Claims 3-5 and 10-13 depend from the method of claim 1, and claims 26-30 depend from claim 25.

Restriction

Applicant has cancelled claims 2, 14-24, and 31-36 without prejudice as directed to a non-elected invention.

Objections

Claims 3 and 26 are objected to because they recite the names of two bacteria *Bifidobacteria* and *Leuconostoc* with incorrect spellings and recite the name of the genus *Streptococcus* twice. These informalities are corrected by the present amendment.

Claim 10 is objected to because it is unclear to the Examiner as to, whether the agents for permeabilization are claimed simply in the alternative or as a Markush group. The claim has been amended to clarify the use of Markush language.

Applicant submits that the amendments to the claims are responsive to the objections and therefore requests that the objections be withdrawn.

35 U.S.C. § 112, Second Paragraph

Claim 12 is rejected as indefinite for lack of antecedent basis for the term "detergent." The claim has been amended to depend from claim 10, thus providing antecedent basis for the term. In view of the amendment, applicant requests that this rejection be withdrawn.

35 U.S.C. §103

Claims 1, 3-5, 10-13, and 25-30 are rejected as allegedly obvious over Somkuti et al. (Enzyme Microb. Technol., 1994, 16:573-576; Somkuti et al. (a)), Somkuti et al. (Curr. Microbiol., 1998, 36:202-206; Somkuti (b)), Herman et al. (In: Streptococcal Genetics, Ferretti and Curtiss (eds.), pages 225-228, 1987), VanBelkum et al. (J. Bacteriol., 1991, 173:7934-7941), Lee et al. (Biotechnol. Bioeng., 1996, 52:572-578), and Chang et al. (U.S. Patent No. 5,766,907). Applicant respectfully traverses the rejection.

The references cited by the Examiner cannot be combined because the Examiner has failed to satisfy the prerequisite for combining references. According to the Federal Circuit:

> The consistent criterion for determination of obviousness is whether the prior art would have suggested to one of ordinary skill in the art that this process should be carried out and would have a reasonable likelihood of success, viewed in the light of the prior art. ...(Citations omitted). Both the suggestion and the expectation of success must be found in the prior art, not in the applicant's disclosure. *In re Dow Chemical Co.*, 837 F.2d 469, 473 (Fed. Cir. 1988) (emphasis added)

Absent such a suggestion to in the art and expectation of success, a *prima facie* case of obviousness is not established. Nowhere does the Office Action point out any suggestion or motivation to make the present invention beyond that surmised by the Examiner. Furthermore, the Office Action does no more than cite references that disclose the individual parts of the invention. For example, the Office Action states "[o]ne of ordinary skill in the art would have had a reasonable expectation of success since all the above references have demonstrated partially each part of the invention separately. (¶) Therefore the above invention would have been *prima facie* obvious to one of ordinary skill in the art (Office Action at page 8). This is not in accordance with established law as stated, for example in *Northern Telecom, Inc. v. Datapoint Corp.*, 908 F.2d 931, 15 USPQ2d 1321 (Fed. Cir. 1990), where the court stated,

> It is insufficient that the prior art disclose the components of the patented device, either separately or in some other combination; there must be some teaching, suggestion or incentive to make the combination made by the inventor.

As discussed above, the invention relates to a permeabilized lactic acid bacterium that contains a heterologous BG gene such that the bacterium has BG activity of at least 4,000 M.U., and a method to make a permeabilized lactose microcarrier. All of these elements must be combined to make the invention. None of the references suggests the combination of all of these features. In fact, the prior art shows a long felt but unresolved need for improvements in the field of lactose hydrolysis. Those of skill in this field seem to have taken two different paths – either using bacteria that naturally produce BG and permeabilizing those bacteria, or by culturing yeast, isolating BG from the yeast and using it as required. However, in the many years that people have been trying to find better ways to remove lactose in dairy products, no one other than applicant hit upon applicant's claimed invention, which is a simple, effective, and elegant solution to the problem. The Office Action attempts to build a rejection based on multiple components, but nothing in the prior art suggests the combination proposed by the Examiner in hindsight.

Several of the references are apparently cited because they disclose permeabilized bacteria (i.e., Somkuti et al. (a), Somkuti et al. (b), and VanBelkum et al.). However, none of these references disclose the use of a recombinant heterologous BG gene under the control of a promoter in any cell, much less in a lactic acid bacterium. Furthermore, none of these references disclose cells having BG activity of at least 4,000 M.U.

Other references were apparently cited because they discuss a cloned BG gene. This group of references includes Herman et al. and Lee et al. Herman et al. describes plasmids that are indigenous to *S. thermophilus* and cloning into *E. coli* of a BG gene from *S. thermophilus*. Herman et al. did not make a lactic acid bacterium containing a recombinant BG gene under the control of a promoter. Nor does the reference disclose a permeabilized lactic acid bacterium, nor does it disclose BG production in a lactic acid bacterium of at least 4,000 MU.

In addition, applicant points out that Herman et al. does not appear to have contemplated the present invention since the purpose of cloning a BG gene into *E. coli* was, according to the reference, "for potential incorporation as a selection marker in an *S. thermophilus* plasmid cloning vector" (Herman at page 22, second column, first full paragraph). Herman et al. never actually introduced this plasmid into *S. thermophilus* because there were no appropriate gene transfer systems, and "there are no published accounts of plasmid transformation in *S. thermophilus* (at page 228, right column).

Lee et al. discloses the production of high levels of BG in an *E. coli* transformed with a plasmid comprising a BG gene under the control of a nar promoter. The Office Action cites Lee et al. as providing construction of a vector which functions in *E. coli* and the induction of expression of BG in transformants (Office Action at page 6).

This reference does not describe the use of a lactic acid bacterium or the permeabilization of the cells. There is no evidence whatsoever that the nar promoter would work in a lactic acid bacterium. In fact, since *E. coli* are gram-negative bacteria and lactic acid bacteria are gram-positive bacteria, there is no reason to believe that one in the art would have been motivated to use a nar promoter for expression of BG in a lactic acid bacterium.

Furthermore, although the Office Action builds a case for obviousness employing Lee et al. (Office Action at page 7), the Office Action (at page 9) also states

> The promoter as taught by Lee et al. is induced by nitrate salts under anaerobic conditions. However, the use of "nar" promoter may not be amenable in processing methods of milk and milk products which is aimed for human consumption. Furthermore, the extra amounts of nitrate that remain in the product will have to be removed which calls for an extra step in the manufacture and increase the cost of production.

Thus, even the Office Action provides reasons why the disclosure by Lee et al. is unlikely to be applied by one in the art to make the present invention.

Finally, the Office Action cites Chang et al. Applicant does not understand why this reference was cited. This embodiment of the invention is not claimed at the present time. Even if such an embodiment were claimed, there is nothing in this reference that has anything to do

with a lactic acid bacterium containing recombinant BG gene under the control of a promoter that has BG activity of at least 4,000 M.U., nor does it discuss a permeabilized bacterium.

Applicant does not believe that the cited references suggest the method (e.g., of claim 1) or the bacterium (e.g., of claim 25), much less the methods or cells of the dependent claims. There is no evidence except for the opinion expressed by the Office Action that the cited combination of references would have made the invention obvious. Further, applicants have reason to doubt the validity of the opinion. For example, the Office Action states

> It also appears that the use of agents such as detergents and ethanol was also well known in the art for permeabilization of lactic acid bacteria. The art also teaches the cDNA clone for *S. thermophilus* BG and that the enzyme from *S. thermophilus* was most favored in the industry. It also appears that there has been several attempts in the art to produce BG at high levels for using it for different purposes. This is clearly evident from the reference of Lee et al. who demonstrate the production of up to 36,000 MU of BG enzyme.

Applicant does not agree with the statement "the enzyme from *S. thermophilus* was most favored in the industry." The industry generally uses BG derived from a yeast, *Kluyveromyces lactis*, not BG from a lactic acid bacterium. For example, Somkuti et al (b) (at page 202, column 2, first full paragraph) states "[a]t present, low-lactose milk is produced primarily with β-gal isolated from yeast (*Kluyveromyces* sp.)..." Applicant respectfully submits that the statement in the Office Action calls into question other unsubstantiated generalizations made in the Office Action regarding obviousness to one of skill in the art.

The Office Action improperly strings together the cited references without providing any suggestion or motivation to combine these references. For example, the Office Action (at page 7) states

> It would have been, obvious to one of ordinary skill in the art, especially those interested in developing lactic acid bacteria, in view of its food-grade status, to develop a method for producing them as microcarrier for BG enzyme. While permeabilizing methods were known, as Somkuti et al. teach, it would have been obvious to one of ordinary skill in the art to use a detergent or ethanol (to avoid the residual effects of the detergent) to

> permeabilize the lactic acid bacteria. Combining the references of Somkuti et al. with that of VanBelkum et al. it would have been obvious to one of ordinary skill in the art to use *L. lactis* for permeabilization and introducing the cDNA encoding BG as it produces lactococcin A which is known to increase permeability of the cells which would be an added advantage to permeabilization by ethanol. It would have been obvious to combine the reference of Herman et al. with the above references and use the cDNA clone provided by Herman et al. to transform *L. lactis* of VanBelkum to obtain a recombinant *L. lactis* with a BG gene from *S. thermophilus* which is most preferred by the industry.

This is simply a string of connections of component parts cobbled together with no explanation of any motivation for such a combination of elements.

The Office Action goes on to state

> It would have been further obvious to combine the reference of Lee et al. with all the above references to make a construct comprising the BG gene from *S. thermophilus* ---because of its ability to be active at slightly elevated temperatures--- operatively linked to "nar" promoter and use such a vector to transform *L. lactis* such that the resulting recombinant *L. lactis* produced very high levels of thermophilic BG anywhere from 4,000 to more than 10,000 MU. Using such a transformed *L. lactis* it would have been obvious to permeabilize it either using the detergents or ethanol as taught by Somkuti et al. to obtain a lactic acid bacterial strain which is capable of producing very high levels of BG with high permeability such that it would be an ideal product as a microcarrier for BG to hydrolyze lactose in milk and milk products.

As discussed above, there is no evidence that the nar promoter would work in a lactic acid bacterium much less be expected to promote expression of high BG levels. The Office Action then takes its hypothetical bacterium containing a promoter that has never been shown to work in a lactic acid bacterium or function to promote expression of a BG gene and confers it with the ability to produce high levels of BG. Having done this, the Office Action then permeabilizes the bacterium declaring it an ideal microcarrier for BG.

Applicant respectfully submits that the Examiner has inadvertently succumbed to the trap of hindsight reconstruction. The assertion that the combination of references cited in the Office Action not just could be combined, but would have been combined, by one in the art to make the claimed invention can only be based on impermissible hindsight, as there is nothing in the cited art which would suggest modification of any of the substituted references to obtain the claimed invention. The Court of Appeals for the Federal Circuit made this clear in W.L. Gore & Associates v. Garlock, Inc., 721 F.2d 1540, 1553, 220 USPQ 303, 312-13 (Fed. Cir. 1983), cert. denied, 469 U.S. 851 (1984):

> To imbue one of ordinary skill in the art with knowledge of the invention... when no prior art reference or references of record convey or suggest that knowledge, is to fall victim to the insidious effect of a hindsight syndrome wherein that which only the inventor taught is used against its teacher.

As part of the alleged string of connections between references, the Office Action states at page 8 (emphasis added)

> Next, it would have been obvious to one of ordinary skill in the art to combine all the above references with that of Chang et al. to package the highly permeable, and high BG producing *L. lactis* in immobilized form using calcium alginate method such that the recombinant bacteria can be transported and stored to be used where and when required. One of ordinary skill in the art would have been motivated to do the above since there is a demand for agents which can be ingested safely and at the same time hydrolyze lactose in milk and milk products by lactose intolerant people and also due to the fact that the *S. thermophilus* enzyme, with its relatively high (50-55 degree C) optimum temperature could replace less-heat-tolerant beta-galactosidase preparations derived from yeasts that are currently used on a commercial scale. One of ordinary skill in the art would have had a reasonable expectation of success since all the above references have demonstrated partially each part of the invention separately.

Even if it would have been obvious to one of skill in the art that "there is a demand for agents that can be ingested safely and at the same time hydrolyze lactose in milk and milk products by lactose intolerant people [sic]," it is not explained how one in the art would have combined all of

the references, none of which suggest the claimed method or cells, to make the claimed invention.

The disparate nature of the references is evidenced by the Office Action itself, which states that all of the references "have demonstrated partially each part of the invention separately" (at page 8, emphasis added).

In view of the arguments presented above, applicant does not believe that the Office Action presents a *prima facie* case for obviousness under 35 U.S.C. § 103.

Applicant's Invention Fulfills a Long-Felt Need and Provides Unexpected Results

Even if the Office Action presented a *prima facie* case, which it does not, applicant's invention demonstrates indicia of non-obviousness, e.g., long-felt need in the art and unexpected results.

It is apparent from some of the cited references that there was a need in the art to improve methods for hydrolyzing lactose in dairy products. For example, Somkuti et al. (a) and Somkuti et al. (b) attempt to improve hydrolysis by permeabilizing a lactic acid bacterium. However, as discussed above, none of the references alone or in combination suggest the solution provided by the present invention.

Applicant has demonstrated that his invention, a permeabilized lactic acid bacterium containing a recombinant BG linked to a promoter, can hydrolyze lactose at an unexpectedly fast rate compared to wild-type lactic acid bacteria (e.g., specification at page 25, lines 2-6). This is evidenced by the fact that such bacteria can hydrolyze lactose in milk in less than 6 hours at 4^0C. This ability to efficiently hydrolyze lactose within such a short incubation time and at low temperature contrasts with current methods in the industry in which enzyme incubations are generally carried out at 8-10°C for 18-24 hours.

The present invention provides not only the unexpected result of greater efficiency at low temperature, but also the distinct advantage of incubation at low temperatures, which can streamline the production of lactose-free milk by reducing incubation time and allowing production in a single operating day. In addition, it is known in the art related to dairy processing that psychotrophs, bacteria that grow at cold temperature, can adversely affect the flavor of milk. Conventional BG treatments can be used at low temperature, however, they

require long incubation times (e.g., 16-24 hours), which reduce production efficiency and permit psychotroph growth. The invention provides an unexpectedly efficient method of hydrolyzing lactose such that the hydrolysis can be carried out at low temperature, but still in a relatively short time. This can reduce psychotroph growth, thereby reducing the chance of such organisms causing an off-flavor to BG-treated milk. The invention therefore fulfills a need in the art and provides unexpected advantages.

In view of the arguments presented above, applicant submits claims 1, 3-5, 10-13, and 25-30 are not obvious and respectfully requests withdrawal of the rejection under 35 U.S.C. § 103 be withdrawn.

Rejection of claims 6-9

Claims 6-9 are rejected under 35 U.S.C. 103 (a) as being unpatentable over all of the references discussed above (Somkuti et al. (a), Somkuti et al. (b), Herman et al., VanBelkum et al., and Lee et al.) as applied to claims 1 and 3-5, 10-13, 25-30 above, and further in view of Kuipers et al. (U.S. Patent No. 5,914,248). Applicant respectfully traverses the rejection.

Claims 6-9 are drawn to the use of a promoter for expression of BG enzyme in the recombinant lactic acid bacteria, such that the promoter is operatively linked to a BG gene. The promoter can be from a gene encoding an antimicrobial peptide or a lantibiotic such as a nisin gene promoter, nisA. The Office Action constructs an obviousness rejection of claims 1 and 3-5, 10-13, 25-30, as discussed and refuted by applicant above. Applicant believes that he has provided ample argument to justify withdrawal of the obviousness rejection of the independent claims, including claim 1, from which claims 6-9 depend. Applicant points out that "[d]ependent claims are nonobvious under section 103 if the independent claims from which they depend are nonobvious." *In re Fine*, 5 U.S.P.Q.2d 1596, 1600 (Fed. Cir. 1988) (emphasis added). Since, as argued above, claim 1 is not obvious, claims 6-9 cannot be obvious.

Even if the arguments supporting withdrawal of independent claim 1 were not adequate, the addition of Kuipers et al. cannot make up for the deficiencies of the other cited references. The Office Action seems to argue that one in the art would find Lee et al. but find it deficient. To make up for the deficiencies of Lee et al., one in the art would look to Kuipers et al.

Kuipers et al. discloses a method for gene expression in lactic acid bacteria by providing a DNA fragment that is under the control of a promoter for a nisA promoter of the nisin gene (an antimicrobial peptide) from *L. lactis*. The Office Action describes a number of "advantages" of using the nisA promoter. However, the reference does not describe the use of recombinant BG gene under the control of a promoter such as nisA or that such cells have BG activity of at least 4,000 MU, nor does the reference discuss permeabilization of cells.

The Office Action states that

> it would have been obvious to one of ordinary skill in the art to replace the "nar" promoter taught by Lee et al.... with the nisA promoter of nisin gene. One of ordinary skill in the art would have been motivated to do so as Kuipers et al. teach that a highly controlled induction of the heterologous protein, using very small amounts of nisin can be obtained with nisA promoter. One of ordinary skill in the art would also be motivated to use nisA promoter as it would not add the extra step of removing the "nar" inducer "nitrate" during the manufacture of milk and milk product. One of ordinary skill in the art would have a reasonable expectation of success as Kuipers et al. demonstrate the over expression of two gene products, beta-glucuronidase and aminopeptidase, using the nisA promoter and very small amounts of nisin as an inducer.

Applicant respectfully disagrees.

As discussed above, there is no reason to believe that one in the art would have turned to Lee et al. to make the present invention since Lee et al. discusses the use of an *E. coli* promoter in *E. coli*, a gram-negative bacterium, and makes no suggestion that the nar promoter would work in a gram-positive bacterium, much less a lactic acid bacterium. In keeping with the reasoning of the Office Action, if one in the art were never motivated to apply Lee et al. to make the present invention, they would certainly not have turned to Kuipers et al.

Applicant therefore submits that not only is there no suggestion in the art to employ a nisA to promote expression of a BG gene, but the Office Action fails to provide evidence that one in the art would have been motivated to combine the plethora of cited references to make the present invention.

In view of the arguments presented above, applicant respectfully requests that the rejection of claims 6-9 under 35 U.S.C. § 103 be withdrawn.

CONCLUSION

Attached is a marked-up version of the changes being made by the current amendment.

Applicant submits that all claims are in order for allowance, which action is requested. Enclosed is a $55.00 check for the Petition for Extension of Time fee. Please apply any other charges or credits to Deposit Account No. 06-1050, referencing attorney docket no. 11072-002001.

Respectfully submitted,

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Version with markings to show changes made

In the claims

Claims 3, 10, 12, and 26 have been amended as follows:

3. (Amended) The method of claim 1, wherein the lactic acid bacterium is selected from the group consisting of *Streptococcus, Aerococcus, Carnobacterium, Enteroccus, Erysipelothrix, Gemella, Globicatella, Lactobacillus, Lactococcus, [Bidobacteria] Bifodobacteria, [Leuconostoccocus] Leuconostoc, Pediococcus,[Streptococcus,] Tetragenococcus,* and *Bagococcus bacteria.*

10. (Amended) The method of claim 1, wherein the bacterium is permeabilized by an agent selected from the group consisting of a chemical, a solvent, [or] and a detergent.

12. (Amended) The method of claim [9] 10, wherein the detergent is selected from the group consisting of deoxycholate, sodium dodecyl sulfate, rhamnolipid, and chenodeoxycholate.

26. (Amended) The permeabilized bacterium of claim 25, wherein the bacterium is selected from the group consisting of *Streptococcus, Aerococcus, Carnobacterium, Enteroccus, Erysipelothrix, Gemella, Globicatella, Lactobacillus, Lactococcus, [Bidobacteria] Bifidobacteria, [Leuconostoccocus] Leuconostoc, Pediococcus, [Streptococcus,] Tetragenococcus,* and *Bagococcus bacteria.*